Formation of urso- and ursodeoxy-cholic acids from primary bile acids by *Clostridium absonum*

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Abstract Eight strains of *Clostridium absonum* were shown to form ursocholic acid (UC) from cholic acid (C) and ursodeoxycholic acid (UDC) from chenodeoxycholic acid (CDC) but did not transform deoxycholic acid (DC) in whole cell cultures. The structures of UC and UDC were verified by mass spectroscopy, and by thin-layer chromatography using Komarowsky's spray reagent. The organism transformed C and CDC at concentrations below $1.5 \cdot 10^{-3}$ M and $5.0 \cdot 10^{-4}$ M, respectively; higher concentrations were inhibitory. Optimal yields of the final products were realized at about 15–22 hr and 9–15 hr of incubation, respectively, and were in the range of 60–70%. Additionally, the 7 keto-derivatives, 7 keto-deoxycholic acid (7K-DC) or 7 keto-lithocholic acid (7K-LC) were also formed from C and CDC. With longer periods of incubation, increasing yields of 7K-DC and 7K-LC and decreasing yields of UC and UDC were observed. These time course studies suggest that 7K-DC and 7K-LC are intermediates in the formation of UC and UDC from the primary bile acids. We propose the occurrence of C ⇌ 7K-DC ⇌ UC and CDC ⇌ 7K-LC ⇌ UDC with increasing dominance of back reaction of the second step on aging of the culture. When the initial pH value of the medium was manipulated within the range of 5.8–9.0, increasing yields of UDC from CDC were obtained at higher pH values (maximum yield at pH 9.0 was 83%), with total inhibition of growth and transformation at pH 5.8. In contrast, UC was produced from C at all pH values studied, with marginal differences in yields (maximum yield at pH 8.0 was 50%). In all cases, formation of UC from C was much slower than that of UDC from CDC. In contrast, *C. paraperfringens* transformed none of the above bile acids. We propose that *C. absonum*, or a biochemically similar species, may be present in the human gut and give rise to UDC (and UC) in vivo.—**Macdonald, I. A., D. M. Hutchison, and T. P. Forrest.** Formation of urso- and ursodeoxycholic acids from primary acids by *Clostridium absonum*. *J. Lipid Res.* 1981. **22:** 458–466.

Supplementary key words 7α-hydroxyl group isomerization · 7β-hydroxyl group formation

Various intestinal organisms are known to oxidize the 7α-hydroxyl moiety of primary bile acids (1–5) or to reduce (or 7-dehydroxylate) this same moiety (1, 6–9). Ursodeoxycholic acid (UDC), a 7β-OH-bile acid, occurs naturally in the bile of certain species of bears (10). This bile acid has been shown to arise in significant biliary amounts when gallstone patients are treated with chenodeoxycholic acid (CDC) (11) and in even greater amounts in rabbits when fed CDC after surgical removal of the cecum and appendix (12). However, the mechanism(s) of isomerizing the 7α-hydroxyl group to the β-configuration, giving rise to ursodeoxycholic acid (UDC) and ursocholic acid (UC) in the gut, are not fully understood. There is some experimental evidence that *a*) keto-bile acids arising from microbial oxidation in vivo may be absorbed, transported to the liver by the portal venous system, and subsequently reduced in the liver back to the 7α- or 7β-isomers (13, 14); or *b*) intestinal microorganism(s) may oxidize the 7α-hydroxyl group, then subsequently reduce this group back to the 7β-configuration (15). The following communication describes an organism, *Clostridium absonum*, capable of the latter transformation: the formation of 7β- and 7 keto-bile acids from primary (7α-) bile acids.

The biochemistry of *C. absonum* is remarkably similar to that of *C. perfringens* and *C. paraperfringens*, both well documented "low population" intestinal anaerobes, and is differentiated from them only by a small number of fermentative tests (16). We propose that small populations of these similar organisms may be primarily responsible for the appearance of UDC in the human and animal intestinal tract.

Abbreviations: BHI, brain heart infusion; TLC, thin-layer chromatography; HSDH, hydroxysteroid dehydrogenase; C, cholic acid (3α-, 7α-, 12α-trihydroxy-5β-cholan-24-oic acid); UC, ursocholic acid (3α-, 7β-, 12α-trihydroxy-5β-cholan-24-oic acid); 7K-DC, 7 keto-deoxycholic acid, (3α-, 12α-dihydroxy 7 keto-5β-cholan-24-oic acid); CDC, chenodeoxycholic acid, (3α-, 7α-dihydroxy-5β-cholan-24-oic acid); UDC, ursodeoxycholic acid, (3α-, 7β-dihydroxy-5β-cholan-24-oic acid); 7K-LC, 7-keto-lithocholic acid, (3α-hydroxy-7 keto-5β-cholan-24-oic acid); DC, deoxycholic acid, (3α-, 12α-dihydroxy-5β-cholan-24-oic acid); LC, lithocholic acid, (3α-hydroxy-5β-cholan-24-oic acid).

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MATERIALS AND METHODS

Materials

All strains of *C. absonum* or *C. paraperfringens* either directly or indirectly originated from the laboratory of Dr. S. Nakamura, Department of Bacteriology, Kanazawa University, Kanazawa, Japan. Strains KZ1214 and KZ1217 of *C. absonum* and strains 360, 362, 363, 365, and 366 of *C. paraperfringens* were kindly donated by Dr. Nakamura. Strains 27555, 27636, and 27637 were from the American Type Culture Collection (ATCC). Strains 6903A and 6905A were donated by Dr. L. V. Holdeman of Virginia Polytechnic Institute (VPI). All the VPI and ATCC collection organisms originated from Kanazawa, Japan.

Brain heart infusion (BHI) broth and cooked meat broth were products of Difco Laboratories, Detroit, MI. Of the bile acids, cholic acid (C) was from J. T. Baker Chemicals, Phillipsburg, NJ; deoxycholic acid (DC) from Calbiochemicals, Los Angeles, CA; CDC and UDC from Sigma Chemicals, St. Louis, MO; 7 keto-deoxycholic acid (7K-DC) and 7 keto-lithocholic acid (7K-LC) were from Steraloids, Wiltshire, NH. Methyl-UC was kindly donated by Dr. F. C. Chang, Department of Biochemistry, University of South Alabama. Ether, methanol, chloroform, acetic acid, and parahydroxybenzaldehyde (Komarowsky's reagent) were from Canadian Laboratories, Montreal, Quebec. Liquid scintillation fluid (catalogue #R19229-82) was from British Drug House, Montreal, Quebec. Commercial sheep's blood plates were a product of Becton Dickinson, Cockeysville, MD. Radioactive bile acids $^{14}C_{24}$-labeled C and $^{14}C_{24}$-labeled CDC were obtained from New England Nuclear, Lachine, Quebec. Labeled intermediates $^{14}C_{24}$-labeled 7K-LC and $^{14}C_{24}$-labeled 7K-DC were made from *E. coli* 7α-HSDH (2) and purified by TLC (17). The $^{14}C_{24}$-labeled C and $^{14}C_{24}$-labeled CDC and unlabeled substrates were demonstrated to be more than 98% pure by TLC.

Growth of *C. absonum*

Organisms were grown initially in freshly boiled cooked meat broth for about 12 hr at 37°C and subsequently stored at 4°C and used for no more than 3 weeks as a starter culture source. Unless otherwise designated, freshly boiled or freshly autoclaved BHI broth was used for subsequent growth of organisms. Starter cultures were grown in 10-ml volumes by inoculating freshly boiled BHI broth with 0.5 ml (5% inoculum) of cooked meat culture and incubating overnight. Five-ml culture volumes containing $2 \cdot 10^{-4}$ M CDC or $5 \cdot 10^{-4}$ M C plus approximately 0.003 μCi of $^{14}C_{24}$-labeled CDC or $^{14}C_{24}$-labeled C, respectively, were grown for periods of 12, 24, or 48 hr. Time course studies were performed similarly, except that 50-ml cultures were used and 5-ml sampling volumes were withdrawn at various time intervals. The absorbance at 660 nm of each culture sample was measured in a Beckman DB-GT spectrophotometer equipped with a Beckman 10-inch recorder. Cultures were then frozen at −20°C until extraction.

Extraction procedure

Whole cultures were thawed, adjusted to pH 3 with 1M HCl, and twice extracted with 5.0-ml volumes of ether in centrifuge tubes equipped with ground-glass stoppers. Combined ether phases were evaporated to dryness in a fume hood.

Thin-layer chromatography and counting procedure

Seventy-five μl of methanol–water 5:1 (v/v) was added to each tube containing ether extract residue and carefully rinsed down the walls. The entire volume was spotted on a silica gel TLC plate (250 μm thick, medium hard with organic binder) and chromatographed in either system I: chloroform–methanol–acetic acid 40:4:2 (v/v/v) (for cholic acid series) or system II: chloroform–methanol–acetic acid 40:2:2 (v/v/v) (for CDC series). Plates were sprayed with Komarowsky's reagent as described earlier (16), and the spots corresponding to the 7β- and 7 keto-transformation products as well as starting material were scraped. The scrapings were transferred to Pasteur pipettes plugged with cotton wool and eluted with 1 ml of ether–methanol 50:50 (v/v) into counting vials. Ten ml of liquid scintillation fluid was added and the vials were counted for 30 min through a full ^{14}C window in a Nuclear Chicago Mark II liquid scintillation counter. Counts were corrected for quenching.

Activity of products with HSDH preparations

Eluates prepared as above from sprayed TLC plates were tested with 3α-, 7α-, and 12α-HSDH preparations both in the forward and backward directions as described before (17, 18).

Mass spectroscopy

Eluates of unsprayed areas of TLC plates (corresponding to specific sprayed areas) were methylated (19) before mass spectroscopy. Mass spectra were obtained on a 21–164 mass spectrometer (Consolidated Electrodynamics Inc.) operating under the following conditions: direct probe temperature, 145°C; source temperature, 150°C; ionization potential, 70eV; accelerating voltage, 800V.

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TABLE 1. Chromatographic properties of *C. absonum* transformation products and standards and their reaction with Komarowsky's reagent and group-specific HSDH

Biological Product or Standard	R_f Solvent I	R_f Solvent II	Color with Komarowsky's Reagent	3α-HSDH (*P. testosteroni*) Forward	3α-HSDH (*P. testosteroni*) Reverse	7α-HSDH (*E. coli*) Forward	7α-HSDH (*E. coli*) Reverse	12α-HSDH (*Clostridium* group) Forward	12α-HSDH (*Clostridium* group) Reverse
C (standard)	.16		red-purple	+	+	+	+	+	+
UC (biol.)	.24		rust-orange	+	+	−	−	+	+
7K-DC (standard)	.37		lime	+	+	−	−	+	+
7K-DC (biol.)	.37		lime	+	+	−	+	+	+
CDC (standard)		.25	blue-purple	+	+	+	+	−	−
UDC (standard)		.32	purple-blue	+	+	−	−	−	−
UDC (biol.)		.32	purple-blue	+	+	−	−	−	−
7K-LC (standard)		.51	med. brown	+	+	−	+	−	−
7K-LC (biol.)		.51	med. brown	+	+	−	+	−	−

Viable count estimations

Samples of *C. absonum* broth cultures (at t = 8, 24, and 48 hr) were diluted serially into ice-cold BHI broth and 0.05-ml aliquots were spread over blood agar plates which were incubated anaerobically as described before (5). Colonies were counted after 24 hr incubation.

RESULTS

All eight *C. absonum* strains examined were capable of producing both 7β-hydroxy and 7 keto-transformation products, when presented with primary bile acids. However, none of the ten strains of *C. paraperfringens* transformed bile acids and none of the eight strains of *C. absonum* transformed DC. *C. absonum* strain VPI #6905, when grown to stationary phase, then boiled and incubated with primary bile acids, did not transform either CDC or C. In the remaining part of the Results section, we have further characterized the transformation of C and CDC by *C. absonum* strain VPI #6905.

Identification of 7 urso- and 7 keto-transformation products

As shown in **Table 1,** our TLC solvent systems (see Methods and Materials) clearly separated 7K-DC, UC, and C (system I) and also 7K-LC, UDC, and CDC (system II). In fact, the resolution between CDC and UDC represents a substantial improvement over that described by Yahiro, Setoguchi, and Katsuki (12) using gas–liquid chromatography. As shown in an earlier study (17), the color developed with Komarowsky's reagent was also helpful in distinguishing transformation products. Of the transformation products observed, 7K-LC gave the least intense reaction with the spray reagent and required a heavier spray for clear visualization. No products other than 7β- and 7 keto-transformation products were evident in either case. Additionally, both forward and backward reactions with 3α-, 7α-, and 12α-HSDH preparations were consistent with the structural assignment of the products (Table 1).

Mass spectroscopy

The most significant fragment ions in the mass spectra of the methyl esters of UC and UDC obtained from the *C. absonum* are summarized in **Table 2.** For comparison, the fragmentation patterns of standard samples of the methyl esters of C, CDC, UC, and UDC are also shown. Although each pair of isomers give similar spectra, the very large differences in peak intensities permit easy identification of the individual isomers.

The main difference in the spectra of the isomers is observed in the intensities of the peaks due to the loss of water molecules; the 7-equatorial hydroxyl group of the urso-(7β-OH) isomers loses water less readily than does the 7-axial group of the 7α-isomers. The major fragment ions which reflect this difference are the ions resulting from the loss of water molecules from the molecular ion and those resulting from the combination of a loss of water and the side chain. The complete list of all of these ions is given in Table 2. The comparison clearly confirms the assigned structures of the two *C. absonum* products.

Losses of bile acids by extraction and elution from TLC plates

Approximately 96% ± 2% and 98% ± 1% of labeled C and CDC, respectively, were extracted by the procedure. Elution from silica was quantitative in both cases with less than 0.5% remaining uneluted.

Effects of bile acid concentrations, time, and pH value

As shown in **Fig. 1 a–d,** the organisms were capable of growing at a CDC concentration of 5.0×10^{-4} M

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TABLE 2. Significant fragment ions in the mass spectra of methyl esters of 7α- and 7β-hydroxylated bile acids

Standard or *C. absonum* Product (Me ester of cholan-24-oic acid)	Significant Fragments (m/e)	% Relative Intensity
3α-, 7α-, 12α-trihydroxy-(C) standard	422 $(M)^{+\cdot}$	<1
	404 $(M-H_2O)^{+\cdot}$	5
	386 $(M-2H_2O)^{+\cdot}$	86
	368 $(M-3H_2O)^{+\cdot}$	43
	289 $(M-H_2O-115)^{+}$	10
	271 $(M-2H_2O-115)^{+}$	88
	253 $(M-3H_2O-115)^{+}$	100
3α-, 7β-, 12α-trihydroxy-(UC) standard	422 $(M)^{+\cdot}$	0.5
	404 $(M-H_2O)^{+\cdot}$	4
	386 $(M-2H_2O)^{+\cdot}$	30
	368 $(M-3H_2O)^{+\cdot}$	13
	289 $(M-H_2O-115)^{+}$	100
	271 $(M-2H_2O-115)^{+}$	76
	253 $(M-3H_2O-115)^{+}$	71
3α, 7β, 12α-trihydroxy-(UC) product from *C. absonum*	422 $(M)^{+\cdot}$	<1
	404 $(M-H_2O)^{+\cdot}$	6
	386 $(M-2H_2O)^{+\cdot}$	30
	368 $(M-3H_2O)^{+\cdot}$	16
	289 $(M-H_2O-115)^{+}$	100
	271 $(M-2H_2O-115)^{+}$	85
	253 $(M-3H_2O-115)^{+}$	84
3α-, 7α-dihydroxy-(CDC) standard	406 $(M)^{+\cdot}$	6
	388 $(M-H_2O)^{+\cdot}$	37
	370 $(M-2H_2O)^{+\cdot}$	100
	273 $(M-H_2O-115)^{+}$	32
	255 $(M-2H_2O-115)^{+}$	24
3α-, 7β-dihydroxy-(UDC) standard	406 $(M)^{+\cdot}$	16
	388 $(M-H_2O)^{+\cdot}$	89
	370 $(M-2H_2O)^{+\cdot}$	100
	273 $(M-H_2O-115)^{+}$	32
	255 $(M-2H_2O-115)^{+}$	68
3α-, 7β-dihydroxy-(UDC) product from *C. absonum*	406 $(M)^{+\cdot}$	16
	388 $(M-H_2O)^{+\cdot}$	89
	370 $(M-2H_2O)^{+\cdot}$	100
	273 $(M-H_2O-115)^{+}$	26
	255 $(M-2H_2O-115)^{+}$	62

and a C concentration of 1.5×10^{-3} M, although there was partial growth inhibition. Higher bile acid concentrations inhibited growth, yet a measurable amount of transformation was shown to occur at inhibitory bile acid concentrations (Fig. 1). Yields of UC and UDC were optimal when C and CDC concentrations were in the range of $0.5-1.5 \times 10^{-3}$ M and $1.0-5.0 \times 10^{-4}$ M, respectively. Substrate concentrations above or below this range gave consistently lower yields. The yields of 7K-DC and 7K-LC were substantially higher when the bacteria were harvested after 48 hr incubation (Figs. 1b and d). In contrast, the yields of UC and UDC were higher after 24 hr incubation (Figs. 1a and c).

Time course curves (**Fig. 2a and b**) showed that: *a*) CDC apparently reacts much faster than C, giving rise initially to a greater quantity of 7 keto-intermediate; *b*) optimal levels of UDC are achieved in a comparable time frame as those of UC; and *c*) if C- or CDC-containing cultures were left for a prolonged period (48 or 24 hr, respectively), increasing amounts of 7K-DC and 7K-LC were formed, apparently at the expense of UC and UDC, (verifying a similar observation for Fig. 1). Additionally, during the initial phases of growth (3–12 hr) a great deal of evolution of gas was apparent.

In **Fig. 3,** the effects of adjusting the initial pH of the medium within the range 5.8–9.0 are shown. Optimal yields of UDC from CDC (as much as 83%), with only trace amounts of 7K-LC, were obtained at pH 9 at 12 hr (Fig. 3c). In contrast, no growth (and no transformation) was obtained at pH 5.8 in the presence of CDC. A similar pH-CDC effect was observed on harvesting at 24 hr (Fig. 3d). However,

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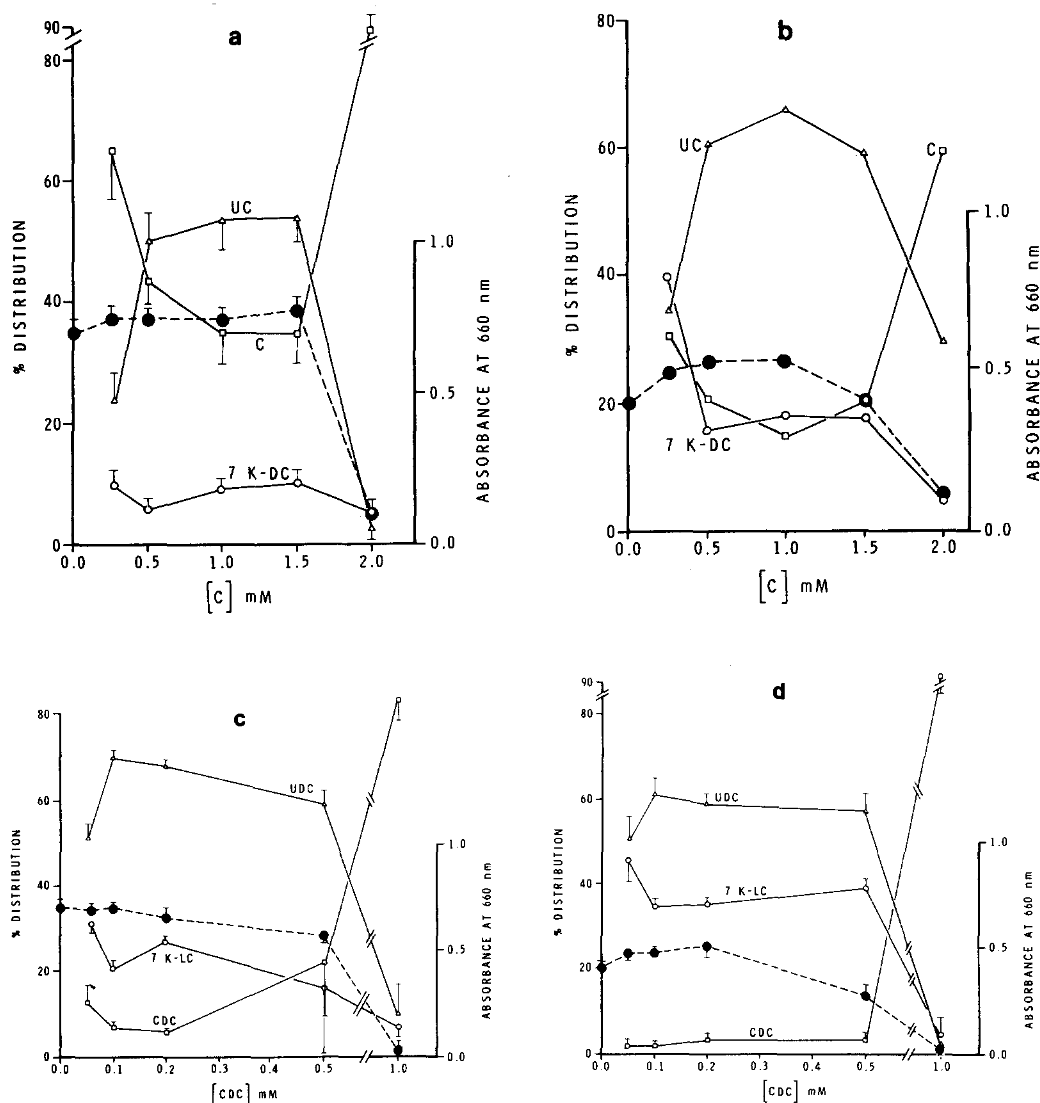

Fig. 1. Effect of initial bile acid concentration on formation of UC and 7K-DC from C by *C. absonum* harvested at (a) t = 24 hr, (b) t = 48 hr; and UDC and 7K-LC from CDC by *C. absonum* harvested at (c) t = 24 hr and (d) t = 48 hr. (□ —— □), primary bile acid substrate; (△ —— △), 7β-transformation product; (○ —— ○), 7-keto transformation product; and (● —— ●), absorbance of culture at 660 nm. (Determination in duplicate ± SD)

at 24 hr, compared to 12 hr, greater yields of 7K-LC and lower yields of UDC were observed, particularly at high pH values. In analogous studies with C as substrate (Fig. 3c and d), there was a less dramatic effect of pH value. In contrast to the studies with CDC, the organisms grew at low pH values in the presence of C (although the growth at pH 5.8 was slow). Optimal yields here (as much as 50%) were obtained at pH 8 and t = 24 hr. Final pH values for the adjusted series (t = 12 hr) were approximately 5.8, 6.2, 6.4, 6.8, and 6.8 for both CDC- and C-containing cultures. (Comparatively little change occurred at low initial pH values).

Estimation of viable counts

Viable count data along with pH and culture absorbance values (660 nm) are summarized in **Table 3.** A ten-fold drop in numbers of viable organisms occurred when cultures were allowed to age from 24 to 48 hr. There appeared to be a small drop in viable count within the period between 8 and 24 hr only when CDC was added to the culture. A parallel drop in absorbance at 660 nm was evident; however, dead cells and cellular debris, which can contribute to the absorbance at 660 nm but not to the viable counts, permits only a rough comparison. A drop in pH value

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from a starting value of about 7.3 to 6.4 was evident after 8 hr. Between 8 and 48 hr, there was a gradual increase to pH 6.7.

DISCUSSION

Clearly, interest in 7α-OH isomerizing organisms such as *C. absonum* stems from: *a*) the ability of the product, UDC, to reduce safely and efficaciously the biliary lithogenic index (20) and dissolve cholesterol gallstones (21, 22) without the metabolic side effects associated with CDC therapy (23, 24), and *b*) the potential ability of such organisms to slow down the rate of 7α-OH dehydroxylation, particularly the formation of LC (a notorious liver toxin (25, 26) and comutagen (27)) from CDC. The product, UDC, 7-dehydroxylates at about one-tenth of the rate of that for CDC (28). Also, UDC was more resistant to the dehydroxylase of *C. leptum* (7, 8) and a Eubacterium species (9) in whole cell studies.

The preliminary studies reported here are consistent with the hypothesis that the 7 keto-bile acids are intermediate to the formation of 7β-bile acids with both steps being reversible. When labeled 7 keto-intermediates are incubated with *C. absonum,* label is transformed both to 7α- and 7β-hydroxylated bile acids.[1] In the time course studies with primary bile acids (Fig. 2), the initial rapid formation of urso-bile acids with relatively small amounts of keto-intermediate present suggests that the first step during the early phase of the culture may become rate-limiting (**Fig. 4**). A second mechanism of formation of urso-bile acid from primary bile acid with no keto-intermediate as proposed by Sarva et al. (15) appears a less likely explanation.

The accumulation of 7 keto-bile acid in the latter part of the time course study, but no further increase in urso-product, could be a result of feedback inhibition by urso-bile acid selectively blocking the second step (7α-HSDH activity). However, there is actually a drop in the level of urso-bile acid (particularly UDC); thus there may be a feedback inhibition by UDC that selectively blocks the forward direction of the second step. A second possibility exists: various reductive activities within the cell may lead to oxidation of the NADPH pool to mostly NADP and this may ultimately cause both primary and urso-bile acids to become oxidized to the 7-keto-derivative. We are currently testing both of these hypotheses.

[1] Macdonald, I. A. Unpublished data.

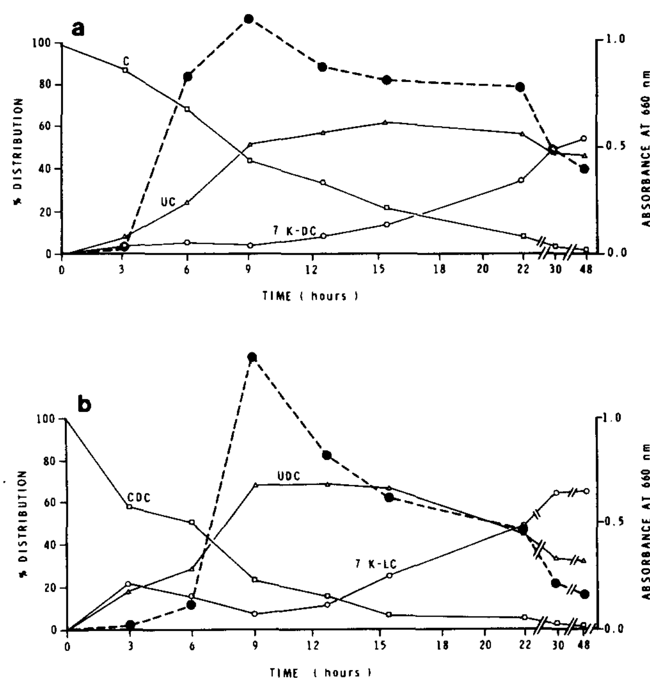

Fig. 2. Time course studies on (a) formation of UC and 7K-DC from C and (b) formation of UDC and 7K-LC from CDC by *C. absonum*. Initial concentrations of C and CDC were $5 \cdot 10^{-4}$ M and $2 \cdot 10^{-4}$ M, respectively, symbols as in Fig. 1. Dotted line designates pH values of medium.

Prolonged exposure of the organism to pH values less than neutrality may be responsible for the drop in the number of viable organisms with time. Acetic, formic, and butyric acids have been demonstrated in spent bacterial medium by gas–liquid chromatography and carbon dioxide has been detected by mass spectroscopy of trapped gas.[1] The reason for the small rise in pH after 8 hr is not clear but could be due simply to loss of CO_2; this pH change can be achieved merely by boiling an 8-hr culture (Table 3). The exact relationship between the drop in pH value (and drop in viable cells) and the accumulation of 7 keto-product remains to be established.

It should be mentioned that in vitro studies on various bile acid hydroxysteroid dehydrogenases demonstrate that greater yields of keto-products were obtained at higher pH values (1–5). However, it is difficult to compare cell-free enzyme studies with whole cell culture studies. In the former, repression of enzyme synthesis need not be considered and the level of oxidized (or reduced) nucleotide can be better controlled. Additionally, cell permeability (in both directions) is not a consideration in cell-free systems.

The faster rate and apparently greater efficiency of transformation of CDC to UDC compared to C

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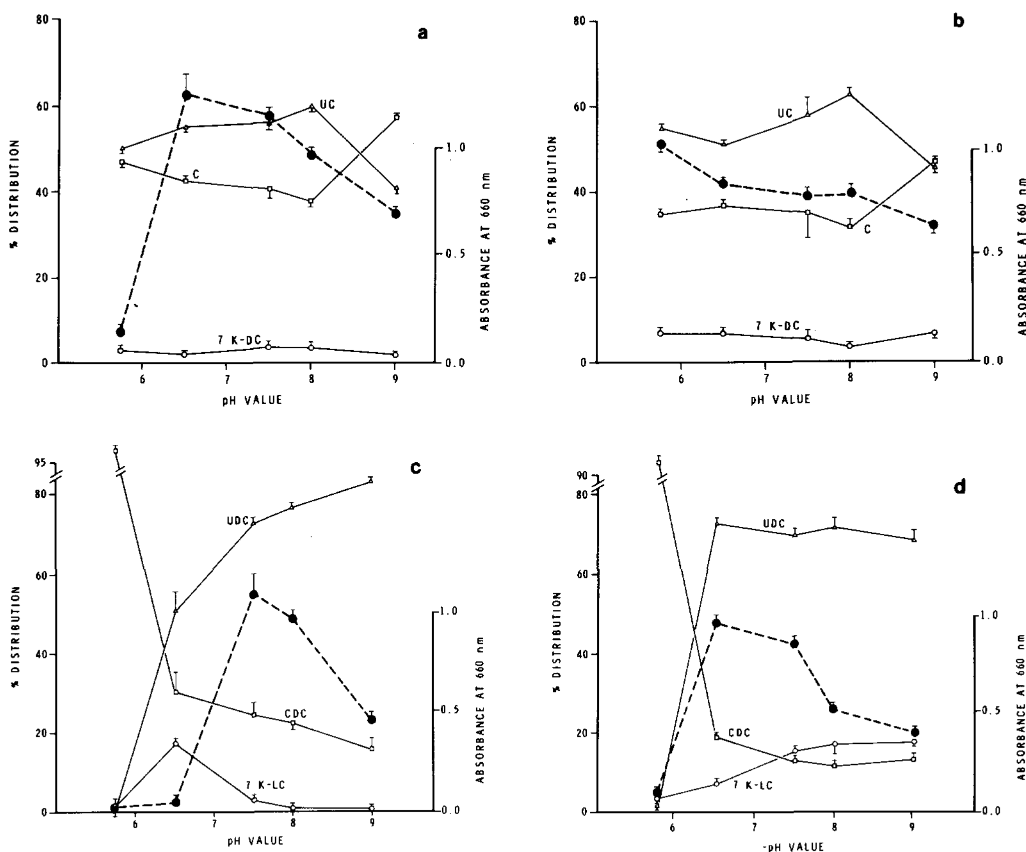

Fig. 3. Effect of pH value on bile acid transformation by *C. absonum* (a) substrate C, harvest time 12 hr; (b) substrate C, harvest time 24 hr; (c) substrate CDC, harvest time 12 hr; (d) substrate CDC harvest time 24 hr. Initial concentrations of C and CDC were $5 \cdot 10^{-4}$ M and $2 \cdot 10^{-4}$ M, respectively. Symbols as in Fig. 1; determinations in duplicate ± SD.

to UC may be due to: *a*) differences in substrate-enzyme affinity; *b*) differences in the ability of the two substrates to induce the enzyme(s); and *c*) differences between C and CDC transformation steps in their equilibrium constants.

These observed rate and yield differences are consistent with the greater amount of UDC (compared to UC) observed as a trace component in human bile (29).

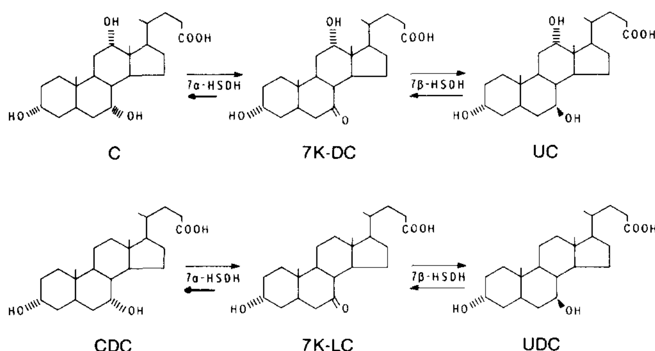

Fig. 4. Reaction scheme for the transformation of (a) C to 7K-DC and then to UC and (b) CDC to 7K-LC and then to UDC.

The reason for some measurable bile acid transformation occurring at high bile acid concentrations where microbial inhibition occurs is not clear. It appears that even low numbers of organisms growing slowly may still have considerable transforming capacity.

We propose that *C. absonum* (or a biochemically similar organism) may be responsible for 7α-OH group transformation in the human gut. *C. absonum* has been isolated from soil (16) and the one attempt to isolate this organism from the intestine has failed (16); thus gut populations of this organism (assuming their existence) are probably very low (<10^4 or 10^3 organisms/gram wet stool). *C. perfringens*, a biochemically and morphologically similar organism usually occurs at approximately 10^4 organisms/gram wet stool (1) but was carried in more than half of a human population sample (30). Increased formation of UDC in patients fed CDC (11) or in appendectomized/cecumectomized rabbits fed CDC (12) is

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consistent with the transformation process being bile acid-inducible.

Current studies, focusing on the metabolism of UDC, 7K-DC, and 7K-LC by this organism, should confirm the proposed pathway. Additionally, we are investigating the properties of bile acid-inducible 7α- and 7β-HSDH activities and formation of UC and UDC in cell-free preparations of *C. absonum*.

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TABLE 3. Effect of harvest time on viable counts[a] (organisms/ml culture), absorbance at 660 nm,[b] and culture pH value[b]

Additions	8 hr			24 hr			48 hr		
	Viable Counts	Absorbance at 660 nm	pH Value	Viable Counts	Absorbance at 660 nm	pH Value	Viable Counts	Absorbance at 660 nm	pH Value
0	1.7 (±0.20)·10^8	0.78 ± 0.03	6.4 ± 0	1.7 (±0.80)·10^8	0.72 ± 0.03	6.6 ± 0.08	1.4 (±0.40)·10^7	0.57 ± 0.006	6.7 ± 0
2·10^{-4} M CDC	1.6 (±0.20)·10^8	0.82 ± 0.02	6.5 ± 0.06	7.6 (±0.11)·10^7	0.56 ± 0.01	6.6 ± 0	8.3 (±0.50)·10^6	0.43 ± 0.02	6.7 ± 0
2·10^{-4} M C	1.7 (±0.30)·10^8	0.75 ± 0.01	6.4 ± 0.10	1.7 (±0.20)·10^8	0.65 ± 0.01	6.6 ± 0.01	2.0 (±0.14)·10^6	0.39 ± 0.00	6.7 ± 0

[a] Determinations in duplicate ±1 SD.
[b] Determinations in triplicate ±1 SD.

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